24. (New) The method of claim 21, wherein the first primer is of SEQ ID NO:1, the second primer is of SEQ ID NO:5 or its RNA equivalent, and the probe is of SEQ ID NO:9 or its RNA equivalent.

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25. (New) The method of claim 21, wherein the first primer is of SEQ ID NO:1, the second primer is of SEQ ID NO:5, and the probe is of SEQ ID NO:27.

26. (New) The method of claim 22, wherein the third primer is of SEQ ID NO:13 or its RNA equivalent, and the oligonucleotide probe that hybridizes to the *abl* sequence is of SEQ ID NO:16 or its RNA equivalent or SEQ ID NO:26.

REMARKS

Claims 1-20 are pending. The claims have been newly rejected. Claims 1 and 9 have been amended and new claims 21-26 have been added. No new matter has been added by these amendments. Support for the amendments is provided throughout the disclosure as described below and in Figures 1A to 1C, Figures 2 and 3, page 12, line 15 to page 13, line 15, page 19, line 25 to page 22, line 6, and Examples 2 to 6 (page 27, line 25 to page 35, line 8). Entry of this amendment and reconsideration of the application are respectfully requested.

Rejections under 35 U.S.C. § 112, Second Paragraph

Claims 4 and 6 have been rejected under 35 U.S.C. § 112, second paragraph, as being indefinite based on the language "stable hybridization complex" because the Examiner stated that the extent of binding was not clearly defined by the term "stable" because "Any interaction at all would be considered better than no interaction" (Paper No. 25, page 2, lines 11-14). Applicants respectfully disagree.

Claims satisfy the requirements of 35 U.S.C. § 112, second paragraph if they define the subject matter with a *reasonable* degree of particularity and distinctness when viewed in light of the content of the application disclosure, the teachings of the prior art, and the claim interpretation that would be given by one possessing the ordinary level of skill in the pertinent art at the time the invention was made (MPEP

2173.02). The use of relative terminology or functional language in a claim does not automatically render the claim indefinite under 35 U.S.C. § 112, second paragraph (MPEP 2173.05(b) and (g)). A term must be evaluated for what it fairly conveys to a person of ordinary skill in the pertinent art in the context in which it is used (MPEP 2173.05(g)).

Applicants submit that the term "stable" when used with "hybridization complex" is an art recognized term which would be clear to a person possessing the ordinary level of skill in the art of molecular biology. One skilled in the art at the time the invention was made would have understood this to mean that a nucleic acid hybridization complex would form by standard hydrogen bonds between complementary base pairs and that the equilibrium conditions would favor maintaining the hybridization complex rather than dissociating the complex into its components.

One skilled in the art at the time the invention was made would have been aware of well known methods of forming and maintaining nucleic acid hybridization complexes in a variety of conditions. This is shown by portions of Molecular Cloning A Laboratory Manual, Second Edition, vol. 1 and 2 (Sambrook et al., Cold Spring Harbor Laboratory Press, 1989) which are attached as Appendix 2. This Manual is widely available and Applicants have selected only a few portions to illustrate what was well known about hybridization technology by 1989, although the references cited in the Manual make clear that this information was well known earlier than that date. Pages 7.37-7.38 disclose a variety of methods that rely on formation of stable hybridization complexes for analysis of RNA (e.g., Northern hybridization, dot and slot hybridization, mapping RNA using probe hybridization and nuclease digestion, solution phase hybridization, and filter hybridization where one component of the hybridization complex is immobilized on a solid support). Pages 9.50-9.51 describe methods and conditions for forming and maintaining hybridization complexes. In particular, item 11 describes how the melting temperature (T_m) of hybridization complexes (RNA:RNA, RNA:DNA and DNA:DNA) can be estimated, and refers to the relative "stability" of such complexes. Pages 11.45-11.49 describe (1) conditions for hybridization of oligonucleotide probes for formation of "stable hybrids" at an acceptable rate, (2) methods for calculating T_m for hybrids between oligonucleotides and their target sequences, (3) estimating the effects of mismatches on stability of hybrids based on their effect on T_m, and (4) conditions for hybridization of pools of oligonucleotides where one or

more oligonucleotides will match the target. Pages 11.55-11.57 describe methods for empirical determination of T_m of an oligonucleotide hybridized to a target sequence. This routine test measures "the temperature at which dissociation of the double-stranded DNA becomes irreversible (T_i) in nonequilibrium conditions that do not favor rehybridization of the released probe to the target." That is, this test determines conditions at which the hybridization complex is maintained and when it is dissociated.

In addition to the information provided in Molecular Cloning A Laboratory Manual, a person of ordinary skill in the art could have relied on other teachings in the prior art that were described in the specification. Prior art that relies on formation of stable hybridization complexes for detection of chromosomal translocations or transcripts from such translocated sequences are described, for example, at page 3, line 19 to page 4, line 6, and page 4, lines 16-26.

Applicants' disclosure would allow a person of ordinary skill in the art to understand the meaning of the term "stable hybridization complex" as used in the context of the claims. For example, a nucleotide sequence is described as a molecule that is able to hydrogen-bond with a complementary DNA or RNA sequence (page 14, lines 4-6). An oligonucleotide includes a sequence of base moieties that provide information permitting the oligonucleotide to hybridize with a complementary nucleic acid strand; and an oligonucleotide may include one or more modified nucleotide base moieties, so long as the oligonucleotide is not sterically prevented from hybridizing with a single-stranded nucleic acid (page 14, line 17 to page 15, line 3). The specification further describes a mediator oligonucleotide which hybridizes in solution to a specific target nucleic acid, thus taking advantage of favorable in-solution hybridization kinetics, and other known methods that rely on formation of stable hybridization complexes (page 26, lines 20 to page 27, line 1). Moreover, the specification includes working examples that describe embodiments in which stable hybridization complexes are formed for isolation of mRNA (e.g., Example 1, at page 24, lines 12-20 and page 24, line 26 to page 25, line 24) and for detection using a probe oligonucleotide (e.g., Example 2 at page 28, lines 16-24).

A person of ordinary skill in the art of molecular biology at the time the invention was made, in view of the prior art, Applicants' disclosure, and the context in which the term occurs would have understood the meaning of the term "stable hybridization complex." Thus, when this term is evaluated for what it fairly

conveys to a person of ordinary skill in the pertinent art in the context in which it is used, it satisfies the requirements of 35 U.S.C. § 112, second paragraph. Therefore, Applicants respectfully request withdrawal of the rejections of claims 4 and 6 under 35 U.S.C. § 112.

Rejections under 35 U.S.C. § 102

Claims 1-5 and 9-17 have been rejected under 35 U.S.C. § 102(b) as anticipated by the disclosure of Sooknanan et al. (Experimental Hematology 21: 1719-1724, 1993).

Sooknanan et al. describe detection of BCR-ABL mRNA by using methods that include nucleic acid sequence based amplification (NASBA) and PCR amplification. Both of the amplification methods described by Sooknanan et al. use serial amplification reactions that require two sets of internal nested oligonucleotide primers that flank the BCR-ABL junction. Sooknanan et al.'s serial NASBA reactions with nested primers include a *first* NASBA reaction that uses a P1 primer (ABL +133 to +150, antisense) and a P2 primer (BCR -178 to -161, sense), and then an aliquot of the first NASBA reaction is added to a *second* NASBA reaction containing primers P1' (BCR -150 to-131, sense) and P2' (ABL +27 to +48, antisense) (page 1719, column 2, paragraph 5 to page 1720, column 1, first paragraph, and page 1721, column 1, paragraph 2). Sooknanan et al.'s serial PCR amplifications use a *first* PCR reaction performed using two primers (ABL +66 to +100 and BCR -292 to-258) and then an aliquot of the first round product is used as the template in the *second* PCR reaction performed using different primers (BCR -250 to -220 and P2' which is ABL +27 to +48) (page 1720, column 1, paragraphs 2-4).

Sooknanan et al. teach detecting the amplified products by using probes specific for BCR-ABL *junctions*. For analysis of the amplified products, Sooknanan et al. teach hybridization using a labeled probe specific for and spanning either the bcr3-abl2 *junction* or the bcr2-abl2 *junction* (page 1720, column 1, last paragraph to column 2, line 7, page 1721, column 2, paragraph 1, lines 5-6, page 1721, column 2, paragraph 3, lines 4-8 and 14-16, and page 1721, column 2, paragraph 5, lines 7-9).

Sooknanan et al. do not describe Applicant's claimed methods for several reasons. First, Sooknanan et al. use nested primers in serial amplification reactions, whereas Applicants' claims do not include steps that use nested primers or serial amplification reactions. This is further supported by

Applicants' disclosure that states:

"It is important to note that this method does not use nested primers and does not require use of serial amplification reactions." (Page 17, lines 20-21.)

Second, Sooknanan et al. use probes that hybridized to the translocation *junction*, i.e., probes that either span the bcr3-abl2 junction or the bcr2-abl2 junction. Sooknanan et al.'s probes are directed to sequences that overlap the splice junction site, whereas Applicants' methods hybridize a probe either of two probe binding sites that do not overlap the splice junction site. This is supported by the disclosure at:

page 18, lines 23-24: "... detection probe may be directed to a nucleotide base sequence located on either side of the splice junction site."

page 18, lines 27-29: "... the probe is not directed to the splice junction, but instead to a flanking sequence located outside the breakpoint or splice region, [therefore] a single probe can detect multiple splice forms."

page 19, lines 1-6: "By 'flanking' the site of translocation is meant that the oligonucleotide binding sites are located at positions on either side of the splice junction" which obviates the need to design splice junction probes for known splice junction sequences or include multiple splice junction probes directed to common and rarer chimeric sequences.

page 19, lines 22-24: "...the probe detects sequences outside of the splice junction (i.e., flanking probes), thus broadening the number of differently spliced oligonucleotides that may be detected using this method."

and page 19, line 29 to page 30, line 3: "... amplicons detected with a probe directed to a nucleotide base sequence flanking the splice junction ...."

Claims 1 and 9 have been amended to make clear that a "single" amplification reaction is used in the claimed methods. The claims already make clear that the detection probe binding site does not overlap or span the splice junction site sequence.

Based on these key differences between Applicants' claimed invention and the disclosure of Sooknanan et al., Applicants respectfully submit that the cited art does not teach each limitation of the claimed invention. Therefore, Applicants request withdrawal of the rejection of claims 1-5 and 9-17 under

35 U.S.C. § 102(b) based on Sooknanan et al.

Claims 19 and 20 have been rejected under 35 U.S.C. § 102(a) as anticipated by the Qiagen Oligotex Direct Protocol for isolation of PolyA+ mRNA (Qiagen product guide, 1/98, page 61, and protocol from the Qiagen web page, www.qiagen.com). The cited Qiagen Product Guide (1998) pages describe "Oligotex" products "for efficient poly A+ mRNA purification." The "Oligotex" product is described at page 61 as "... latex particles that provide maximal surface area for dT_{30} oligonucleotides" and the method of using them is illustrated (Fig. 4.15), which includes "Hybridize mRNA to Oligotex, Collect mRNA:Oligotex complexes, Wash mRNA:Oligotex complexes" and "Elute mRNA from Oligotex." The mRNA isolation procedure is described generally (on page 62):

> "Oligotex purification procedures are convenient and straightforward (Figure 4.15). Oligotex Direct mRNA Kits contain a guanidinium-thiocyanate-based buffer for efficient cell and tissue lysis, protein denaturation, and RNAase inactivation to keep mRNA intact. Homogenized cell or tissue lysates (Oligotex Direct mRNA procedure) or total RNA preparations (Oligotex mRNA procedure) are incubated with Oligotex particles, and Oligotex:mRNA complexes are collected by a brief centrifugation step. After washing, the mRNA is eluted in a small volume of Tris buffer or water. Neither procedure requires precipitation with ethanol."

The Examiner also cited the "Oligotex Direct mRNA Protocol" taken from "Qiagen web page www.qiagen.com". The pages provided (43-47 and 75) describe the "Oligotex Direct mRNA Protocol" in detail and "Buffers for direct mRNA purification from cytoplasm of cultured cells" which presents the "buffer" formulations used in the method. The pages describing the "Oligotex Direct mRNA Protocol" indicate that they are from the "Oligotex Handbook 07/99" (see lower left or lower right corners of pages 43-47 and 75). Applicants understand "07/99" to mean that the protocol was published or made available to the public in July 1999.

Applicants' application was filed on July 23, 1998, and claims the benefit under 35 U.S.C. 119(e) to a provisional application filed on July 23, 1997. The cited Qiagen documents are not prior art to Applicants' invention. Therefore, Applicants respectfully request that the rejection of claims 19 and 20 under 35 U.S.C. § 102(a) be withdrawn.

Application No.: 09/121,239
Filed: July 23, 1998
Art Unit: 1635

AMENDMENT via facsimile
Docket No. GP091-02.UT

Rejections under 35 U.S.C. § 103(a)

To establish a *prima facie* case of obviousness requires: (1) a suggestion or motivation in the prior art to modify the reference or to combine prior reference teachings so that one of ordinary skill in the art would make the claimed composition or carry out the claimed process; and (2) the prior art must also reveal a reasonable expectation of success. The teaching or suggestion to make the claimed invention and the reasonable expectation of success must be found in the prior art, and not be based on applicant's disclosure. *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991), *In re Dow Chemical Co.*, 837 F.2d 469, 473 (Fed. Cir. 1988). When a rejection under § 103 depends on a combination of prior art references, there must be some teaching, suggestion, or motivation to combine the references. *In re Rouffet*, 47 U.S.P.Q.2d 1453, 1456 (Fed. Cir. 1998).

Factual predicates underlying a determination of *prima facie* obviousness include the scope and content of the prior art, the differences between the prior art and the claimed invention, and the level of ordinary skill in the art. *Graham v. John Deere Co.*, 383 U.S. 1, 17-18 (1966); *In re Rouffet*, 149 F.3d 1350, 47 USPQ2d 1453, 1455 (Fed. Cir. 1998). The level of skill in the art cannot be relied upon to provide the suggestion to combine references, i.e., there must be some objective reason to modify or combine the teachings of the references. *Al-site Corp. v. VSI Int'l Inc.*, 174 F.3d 1308, 50 USPQ2d 1161 (Fed. Cir. 1999); *In re Kotzab*, 217 F.3d 1365, 1371, 55 USPQ2d 1313, 1318 (Fed. Cir. 2000). It is impermissible to use hindsight to pick and choose the features of the present invention from the prior art to create the claimed combination. *In re Fine*, 837 F.2d 1071, 5 U.S.P.Q.2d 1596 (Fed. Cir. 1988).

A reference must be considered as a whole and must be considered for all that it teaches. *W.L. Gore & Associates v. Garlock*, 721 F.2d 1540, 1550 (Fed. Cir. 1983). Teaching in the prior art that discourages persons of skill in the art from an approach leading to the claimed invention must be considered and proceeding contrary to accepted wisdom in the art is evidence of nonobviousness. *W.L. Gore & Associates v. Garlock* at 1550; *In re Hedges*, 783 F.2d 1038, 228 USPQ 685 (Fed. Cir. 1986).

If an independent claim is nonobvious under 35 U.S.C. 103, then any claim depending therefrom is nonobvious. *In re Fine*, 837 F.2d 1071, 5 USPQ2d 1596 (Fed. Cir. 1988).

Claims 6-8 have been rejected under 35 U.S.C. § 103(a) as unpatentable over Sooknanan et al. in view of the Qiagen Oligotex Direct Protocol for isolation of PolyA+ mRNA (both cited above).

As discussed above, the cited Qiagen documents are not prior art to Applicants' invention. Therefore this obviousness rejection of claims 6-8 would rely solely on the disclosure of Sooknanan et al. The preparation of RNA method taught by Sooknanan et al. uses a chaotropic compound (guanidinium isothiocyanate), phenol and extractions with chloroform (page 1719, column 2, paragraph 4). As discussed above, Sooknanan et al. teach amplification methods that require internal nested primer sets used in serial amplification reactions, i.e., a first primer set is used in a first amplification reaction and then the product of the first reaction is used as a template for the second primer set used in a second amplification reaction. Also, as discussed above, Sooknanan et al. teach using junction probes that span or overlap the splice junction. All of these disclosures in Sooknanan et al. teach away from Applicants' claimed methods because Applicants' methods (1) do not use extractions using reagents such as phenol or chloroform in preparing RNA, (2) do not use nested primer sets and serial amplification reactions in the amplification step, and (3) do not use probes that overlap the splice junction. Indeed, Applicants' methods proceed contrary to accepted wisdom as described by Sooknanan et al. which is evidence strongly probative of nonobviousness.

Therefore, Applicants respectfully submit that the Examiner has not established a *prima facie* case of obviousness and request that the rejection of claims 6-8 under 35 U.S.C. § 103(a) be withdrawn.

Claim 18 has been rejected under 35 U.S.C. § 103(a) as unpatentable over Sooknanan et al. in view of the Qiagen Oligotex Direct Protocol for isolation of PolyA+ mRNA and further in view of Burg et al. (US Patent 6,300,068 B1). The Sooknanan et al. reference and Qiagen reference were applied to claim 18 as applied to claims 1-17, 19 and 20 discussed above, and the Burg et al. reference was cited for teaching the use of an internal control transcript in methods of nucleic acid amplification, including NASBA reactions. The Examiner concluded that "One of ordinary skill in the art would have had an expectation of success to use internal control sequences in the NASBA methods taught by Sookanan [*sic*] et al. since Burg et al. taught the use of internal control sequences in NASBA reactions." (Paper No. 25, page 11, lines 9-12).

As discussed above the cited Qiagen documents are not prior art to Applicants' invention.

As discussed above, the disclosures in Sooknanan et al. teach away from Applicants' claimed methods because Sooknanan et al. teach use of nested primer sets in serial NASBA reactions and use of detection probes that span the splice junctions of specific translocations. Even if the disclosure of Burg et al. were combined with the teachings of Sooknanan et al., they would not suggest to one skilled in the art to use Applicants' method of claim 18. Applicants' method of claim 18, which depends from claim 9, proceeds contrary to the teachings of Sooknanan et al. which provides evidence of nonobviousness of Applicants' method.

Applicants therefore respectfully submit that the method of claim 18 is not obvious in view of the cited prior art (Sooknanan et al. and Burg et al.). Therefore, Applicants respectfully request that the rejection of claim 18 under 35 U.S.C. § 103 be withdrawn.

New Claims 21-26

New claims 21 to 26 are presented for examination. The independent claim 21 is drawn to a method of detecting a fusion mRNA transcript produced as a result of a human *bcr-abl* translocation, dependent claim 22 is drawn to an embodiment that amplifies and detects a normal *abl* sequence (i.e., not a translocation sequence) as an internal control, and dependent claims 23-26 are drawn to embodiments that use sequences that are described in the Examples of the specification.

Applicants believe that these new claims are consistent with the foregoing remarks about the art cited with regard to pending claims 1-20. Applicants further believe that new claims 21-26 are fully supported by the disclosure and do not add new matter. Applicants respectfully request examination of these claims.

CONCLUSION

In view of the foregoing amendments and remarks, the Applicants respectfully submit that the claims are in condition for allowance. Accordingly, allowance of the application is earnestly solicited. The undersigned has made a good-faith effort to address all the points raised in this Office Action and to place the claims in condition for allowance. If minor matters remain that could be resolved by telephone interview, the Examiner is invited to contact the undersigned at the number below.

Application No.: 09/121,239
Filed: July 23, 1998
Art Unit: 1635

AMENDMENT via facsimile
Docket No. GP091-02.UT

Applicants have submitted a petition herewith for a three-month extension of time to respond to the outstanding Office action and provide authorization that the required fee due in connection with this filing be debited form Deposit Account No. 07-0835.

Respectfully submitted,

Date: June 21, 2002

By: Christine A. Gritzmacher

Christine A. Gritzmacher
Reg. No. 40,627
GEN-PROBE INCORPORATED
Patent Department
10210 Genetic Center Drive
San Diego, California 92121
Tel.: (858) 410-8926 FAX: (858) 410-8928

Enclosures: Appendix 1 (marked up claims)
Appendix 2 (Molecular Cloning A Laboratory Manual pages)

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1. (Amended Four Times) A method for detecting a fusion nucleic acid consisting essentially of the steps of:

a) providing a sample containing a first single-stranded fusion nucleic acid comprising a splice junction;

b) contacting under nucleic acid amplification conditions:

the first single-stranded fusion nucleic acid,

a first primer which hybridizes to the fusion nucleic acid at a first primer binding site located 3' to the splice junction site, and

at least one nucleic acid polymerase activity;

c) amplifying the fusion nucleic acid in [an] a single isothermal nucleic acid amplification reaction using the first primer to produce a plurality of second nucleic acid strands complementary to at least a portion of the first single-stranded fusion nucleic acid that contains the splice junction site, wherein each second nucleic acid strand comprises:

a complementary splice junction site,

a first probe binding site located 3' to and not overlapping the complementary splice junction site, and

a second probe binding site located 5' to and not overlapping the complementary splice junction site, wherein the second probe binding site overlaps or is located 3' to sequence complementary to the first primer binding site;

d) hybridizing the second nucleic acid strands with an oligonucleotide probe under hybridization conditions in which the probe hybridizes to either the first probe binding site or the second probe binding site, thereby forming a probe:target hybrid; and

e) detecting the probe:target hybrid as an indication of the presence of the fusion nucleic acid in the sample.

2. (Reiterated) The method of Claim 1, wherein the first single-stranded fusion nucleic acid is an mRNA, the first primer is a promoter-primer, the polymerase activity comprises an RNA polymerase activity, and the oligonucleotide probe is of the same sense as the mRNA and binds to the first probe binding site.

3. (Reiterated) The method of Claim 1, wherein the first single-stranded fusion nucleic acid is a mRNA, wherein the second nucleic acid strands are complementary RNA, wherein the amplifying step includes contacting the second nucleic acid strand with a second primer or promoter-primer which hybridizes to a second primer binding site located 3' to both the complementary splice junction and the first probe binding site, and wherein the amplifying step uses an RNA polymerase activity, a DNA-directed DNA polymerase activity and an RNA-directed DNA polymerase activity.

4. (Reiterated) The method of Claim 1, wherein the oligonucleotide probe binds to the second probe binding site and does not form a stable hybridization complex with the first single-stranded fusion nucleic acid.

5. (Reiterated) The method of Claim 1, wherein the fusion nucleic acid is a *bcr-abl* fusion mRNA and wherein the oligonucleotide probe binds to a *bcr*-derived nucleotide base sequence in the second nucleic acid strands.

6. (Reiterated) The method of Claim 1, wherein step a) includes preparing RNA from the sample containing the fusion nucleic acid by:

contacting a biological sample comprising the fusion nucleic acid with a solution consisting essentially of:

a buffer,

about 150 mM to about 1 M of a soluble salt,

about 0.5% to about 1.5% (v/v) of a non-ionic detergent, and

a solid support to which is joined an immobilized oligonucleotide comprising a nucleotide base sequence which forms, directly or indirectly, a stable hybridization complex with an RNA under conditions permitting the formation of the stable hybridization complex; and

separating the hybridization complex joined to the solid support from unhybridized sample components without extracting the RNA using reagents such as phenol or

chloroform.

7. (Reiterated) The method of Claim 6, wherein the fusion nucleic acid is mRNA.

8. (Reiterated) The method of Claim 7, wherein the nucleotide base sequence of the immobilized oligonucleotide comprises a poly-T sequence.

9. (Amended Four Times) A method of detecting a fusion mRNA transcript produced as a result of a chromosomal translocation consisting essentially of the steps of:

a) providing a sample containing a fusion mRNA transcript comprising a splice junction;

b) contacting under isothermal nucleic acid amplification conditions:

the fusion mRNA transcript,

a first primer which hybridizes to the fusion mRNA transcript at a first primer binding site derived from a first chromosomal region and located 3' to the splice junction site, and

at least one enzyme having nucleic acid polymerase activity;

c) amplifying the fusion mRNA transcript in a single nucleic acid amplification reaction that uses the first primer to produce a plurality of second nucleic acid strands complementary to at least a portion of the fusion mRNA transcript containing the splice junction site, wherein each second nucleic acid strand comprises:

a complementary splice junction site,

a first probe binding site located 3' to and not overlapping the complementary splice junction site, wherein the first probe binding site is derived from a second chromosomal region, and

a second probe binding site located 5' to and not overlapping the complementary splice junction site, wherein the second probe binding site is derived from a third chromosomal region and overlaps or is located 3' to sequence complementary to the first primer binding site;

d) hybridizing the second nucleic acid strands with an oligonucleotide probe which hybridizes to the second nucleic acid strands at either the first probe binding site or the second probe binding site but does not hybridize to the fusion transcript, thereby forming a hybridization complex of the probe and the second nucleic acid strand; and

e) detecting the hybridization complex as an indication of the presence of the fusion transcript in the sample.

10. (Reiterated) The method of Claim 9, wherein the amplifying step uses only a first primer that is a promoter primer and the enzyme has an RNA polymerase activity, and wherein the hybridizing step uses an oligonucleotide probe which hybridizes to the second nucleic acid at the first probe binding site.

11. (Reiterated) The method of Claim 9, wherein the first probe binding site and the second probe binding site are derived from different locations on the same chromosome in a eukaryotic cell, and the fusion mRNA transcript detected results from an intrachromosomal translocation.

12. (Reiterated) The method of Claim 9, wherein the first probe binding site is derived from a different chromosome than the chromosome from which the second probe binding site is derived, and the fusion mRNA transcript detected results from a translocation involving different chromosomes.

13. (Reiterated) The method of Claim 12, wherein the fusion mRNA transcript results from a translocation of human chromosomes selected from the group consisting of: t(1;19), t(2;5), t(2;13), t(4;11), t(6;9), t(8;21), t(9;11), t(9;22), t(11;14), t(11;19), t(11;22), t(12;21), t(14;18) and t(15;17) translocations.

14. (Reiterated) The method of Claim 13, wherein the fusion mRNA transcript results from a human t(9;22) translocation and the oligonucleotide probe comprises a *bcr*-derived sequence or an *abl*-derived sequence.

15. (Reiterated) One or more oligonucleotides suitable for use in the method of Claim 14, have a

sequence selected from the group consisting of SEQ ID NO:1 to SEQ ID NO:23, SEQ ID NO:26 and SEQ ID NO:27.

16. (Reiterated) The method of Claim 9, wherein the amplifying step uses an RNA polymerase activity, a DNA-directed DNA polymerase activity, and an RNA-directed DNA polymerase activity, and further uses a second primer or promoter primer which hybridizes under amplification conditions to a nucleotide sequence of a complementary RNA produced during the amplifying step.

17. (Reiterated) The method of Claim 16, wherein the RNA-directed DNA polymerase activity and DNA-directed DNA polymerase activity are supplied by a reverse transcriptase.

18. (Reiterated) The method of Claim 9, wherein the amplifying step also amplifies an internal control transcript in the sample by using the first primer and then hybridizing a second oligonucleotide probe which hybridizes to the complement of the internal control transcript but does not hybridize to the complement of the fusion mRNA transcript thereby forming in internal control hybridization complex, and wherein the detecting step also detects the presence of the internal control hybridization complex in the sample, thereby providing an internal standard.

19. (Reiterated) A method of preparing a sample containing RNA suitable for amplification, consisting essentially of the steps of:

a) providing a biological sample comprising unpurified RNA;

b) mixing the biological sample with a solution consisting essentially of:

a buffer at a pH of about 6.5 to about 8.5,

about 150 mM to about 1M of a soluble salt, and

about 0.5% to about 1.5% (v/v) of a non-ionic detergent, to produce a solution containing released RNA;

c) mixing the solution containing released RNA with a solid support to which is joined an immobilized oligonucleotide comprising a nucleotide base sequence which forms a stable immobilized

oligonucleotide:RNA hybridization complex under hybridization conditions;

d) separating the hybridization complex joined to the solid support from unhybridized sample components; and

e) then washing the hybridization complex joined to the solid support with a solution having sufficient salt concentration to maintain the hybridization complex, thereby not requiring extraction using reagents such as phenol or chloroform to prepare RNA.

20. (Reiterated) The method of Claim 19, wherein the biological sample is uncoagulated blood, plasma or bone marrow.

21. (New) A method of detecting a fusion mRNA transcript produced as a result of a human *bcr-abl* translocation comprising the steps of:

a) providing a sample containing a human fusion mRNA transcript comprising a *bcr-abl* splice junction;

b) contacting under isothermal nucleic acid amplification conditions:

the fusion mRNA transcript,

a first primer which hybridizes to the a second primer which hybridizes to the complement of the fusion mRNA transcript at a primer binding site derived from a *bcr* region flanking the *bcr-abl* splice junction site,

a second primer which hybridizes to the complement of the fusion mRNA transcript at a primer binding site derived from a *bcr* region flanking the *bcr-abl* splice junction site,

at least one enzyme having an RNA-directed DNA polymerase activity, and

at least one enzyme having an DNA-directed RNA polymerase activity;

c) amplifying the fusion mRNA transcript in a single nucleic acid amplification reaction that uses

the first primer to produce a second nucleic acid strand complementary to at least a portion of the fusion mRNA transcript containing the *bcr-abl* splice junction site,

the second primer to produce a third nucleic acid strand of the same sense as the fusion

mRNA transcript containing the *bcr-abl* splice junction site, and

the DNA-dependent RNA polymerase activity to produce amplified RNA that is complementary to the fusion mRNA transcript comprising the *bcr-abl* splice junction;

d) hybridizing the amplified RNA with an oligonucleotide probe which hybridizes to a probe binding site derived from a *bcr* region flanking the *bcr-abl* splice junction, thereby forming a hybridization complex; and

e) detecting the hybridization complex as an indication of the presence of the fusion mRNA transcript in the sample.

22. (New) The method of claim 21, further comprising

in the contacting step, contacting a third primer that hybridizes to the complement of a normal *abl* mRNA transcript at a site located downstream of the first primer in an *abl* sequence present in a normal *abl* mRNA transcript that is replaced by *bcr* sequence in the fusion mRNA transcript,

in the amplifying step, amplifying normal *abl* sequence that is replaced by *bcr* sequence in the fusion mRNA transcript,

in the hybridizing step, hybridizing an oligonucleotide probe that hybridizes to a probe binding site derived from an *abl* sequence that is replaced by *bcr* sequence in the fusion mRNA transcript, and

in the detecting step, detecting a hybridization complex made up of the probe hybridized to an *abl* sequence that is replaced by *bcr* sequence in the fusion mRNA transcript, thereby providing an internal control based on amplifying and detecting normal *abl* sequence.

23. (New) The method of claim 21, wherein the first primer is of SEQ ID NO:1, the second primer is of SEQ ID NO:5, and the probe is of SEQ ID NO:9 or SEQ ID NO:27 or is a mixture of SEQ ID NO:9 or SEQ ID NO:27.

24. (New) The method of claim 21, wherein the first primer is of SEQ ID NO:1, the second primer is of SEQ ID NO:5 or its RNA equivalent, and the probe is of SEQ ID NO:9 or its RNA equivalent.

25. (New) The method of claim 21, wherein the first primer is of SEQ ID NO:1, the second primer is of SEQ ID NO:5, and the probe is of SEQ ID NO:27.

26. (New) The method of claim 22, wherein the third primer is of SEQ ID NO:13 or its RNA equivalent, and the oligonucleotide probe that hybridizes to the *abl* sequence is of SEQ ID NO:16 or its RNA equivalent or SEQ ID NO:26.